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Review article

STUDIES ON THE BIOLOGY AND TRAPPING ABILITY OF *NEMATOCTONUSROBUSTUS* (F.R. JONES)

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INTRODUCTION

Nematodes are microscopic multicellular roundworm that inhabit marine, freshwater and terrestrial environment. Some of them are beneficial soil microorganisms that play an important role soil mineralization while others causes plant diseases (Dufour*et al*., 2003).Microbial-feeding nematodes (bacterivores, fungivores, and omnivores) and plant-feeding nematodes are more important in decomposition and nutrient mineralization.. It probably leads to the abundance of the nematodes in environment, their high turnover rate, and the strong interactions with soil microbes. Plant-feeding nematodes are usually live in grass fields and are deleterious to the plant growth, because they can decrease the productivity of the plants with damaging the root systems.

There are several control of plant-feeding nematodes, such as vesicular-arbuscular Mychorrhizal fungi, nematode trapping fungi, or other fungi and bacteria which can prevent the presence of the nematodes (Ingham, 1996). The ability of the nematophagous fungi to grow in the rhizosphere is of great importance for their capacity to control these nematodes. The genus *Nematoctonus* was erected first time by Drechsler in1941 distinguishing it from other nematode destroying fungi by hyphae with clampconnections, a characteristic feature of the Basidiomycotina. The genus *Nematoctonus* is unique in that some species are endoparasitic and some species are predaceous by established criteria. In his original paper Drechsler described *Nematoctonustylosporus* and *Nematoctonusleiosporus*. Seven more species have since be Drechsler been described these are *Nematoctonu shaptocldus* (Drechsler 1946), *Nematoctonu sconcurrens* (Drechsler 1949), *Nematoctonu spachysporus* and *Nematoctonu slaptosporus* (Drechsler 1943), *Nematoctonu scampylosporus* (Drechsler, 1954), *Nematoctonusrobustus* (Jones, 1964) and *Nematoctonu stripotilanius* (Giuma and Cooke 1972). *Nematoctonus* were captured sticky cells born on erect protubence from the prostrate hyphae, occurring at more or less regular intervals. These adhesive process were dumb bell shaped constricted medially. The sticky process were produced in abundance, and captured nematodes after capturing assimilative hyphae penetrated the nematode from the adhesive process, and a dense a mycelial network observed in the nematode body. It has been suggested that *Nematoctonus* species could be used for the biological control of soil born phytonematodes by inducing conidia to infested soil (Cooke 1968). For successful biological control using spores them is required to be satisfied two major conditions i.e. on their addition to the soil they must germinate rapidly to form adhesive organs directly or on their germ tubes. Second conidia their germ tubes or both must remain viable for a sufficiently long period to ensure maximum chance of a host parasitic contact and subsequent infection.

REVIEW OF LITERATURE

History

Nematophagous fungi are carnivorous fungal species that use their spores or mycelial structures to capture vermiform nematodes, or use their hyphal tips to parasitize the eggs and cysts of nematodes (Nordbring-Hertz 2004), or produce toxins to attack nematodes (li *et.al.* 2000) . They are the natural enemies of nematodes and have developed very sophisticated strategies to either infect or capture these nematodes. Nematophagousfingi are a diverse group of microorganisms, and their nematophagous habit is generally considered to have evolved independently in different fungal classes. Traditionally, they are classified into two groups, the nematode trapping fungi and the endoparasites, basedon their parasitic modes on nematodes (Barron, 1977: Gray 1987). Species belonging to thepredatory group produce an extensive hyphal system, and the hold live nematodes. The captured victim is then penetrated, with its entire body contents being consumed rapidly (Barron 1977).

The endoparasites do not produce extensive mycelia but exist as a conidia in the environment and infect nematodes by either adhering to the surface of the prey or direct ingesting on the conidia germinate rapidly and invade entire nematode with assimilative haphae absorbing all the body contents (Gray 1987). As the third group, egg or female parasites are facultative fungi that are common soil saprophytes and areopportunistic isolates obtained from the sedentary stages (female and egg stages) ofsedentary nematodes such as *Heterodera, Globodera, and Meloidogyne,* (Kerry and Jaffee 1997). Some fungi that produce nematode toxins by special structures are proposed to be classified into fourth group (Jansson et.al. 1997; Li et al. 2000). Recently, the fungus *Strophariarugosoannulata* was found to kill nematodes by damaging their cuticles mechanically with a special spiny ball structure, an ancanthocyte, and it could be a representative of a fifth group of nematophagous fungi (Luo et al. 2006).

TAXONOMY

Nine species of *Nematoctonus*have been validly describd (Giuma and Cooke, 1972), and occur in worldwide (Feder, 1962 ; Gazzano, 1978 ; Gray, 1983 ; Kitz and Embree, 1979 ; Mcculloch, 1977). Barron (1978) reported five named and one unknown species of *Nematoctonus* from Ontario region of Canada. Thorn and Barron (1986) were able to recognize twelve *Hohenbuehelia* species to their *Nematoctonus*anamorphs by culturing the basidiospores from fruting bodies or by inducing fruting bodies in culture of *Nematoctonus.*

Nearly 10 species of *Nematoctonus* fungi have been reported, but one species *N.ligniocola* isolated by Solonen and Ruokola (1968), is not validly published since no type was designated. A key to the nine validly published species was published by Giuma and Cooke (1972). Since then, two new species were described under provisional names (Alger, 1980). The genus *Nematoctonus* is unique that some species are endoparasitic and some are predaceous. The endoparasitic species are *N.leiosporus, N.tylosporus, N.leptosporus* and *N.pachysporus*. In endoparasitic species infection is initiated by means of adhesive conidia adhesive knob is produced at distal end of each conidium which is adhere to host cuticle, after attachment penetration peg enters into the host and proliferate inside body cavity and consuming the entire contents. Predaceous *Nematoctonus* species *are N.concurrens, N.haptocladus, N.tripolitanius, N.campylosporus and N.robustus*. In these conidia are produced on hyphae outside the nematode. These conidia do not produce adhesive knobs instead the knobs produced on external hyphae ramifying from body of the nematode. These adhesive cells capture additional nematodes, produce more hyphae with adhesive cells.

SURVEY

A large number of workers have worked on the distribution of nematophagous fungi (endoparasitc and predacious fungi) and have arrived to the conclusion that they are widely distributed. They are more frequently encountered from leaf litter, decaying woods, dungs and freshly decaying plant's foliage (Duddington, 1940-1952; Soprunov, 1958; Drechsler, 1937-1941; Zwirn-Hirsch, 1947 Shepherd, 1955; Maupas, 1915). Very few attempts have been made by workers to study the distribution of *Nematoctonus* from different regions. Survey conducted by Drechsler 1941, Giuma and Cooke 1971, Barron 1978, Gray 1984 Thorn and barron 1986 Stadler*et.al*.1994.concluded that species of *Nematoctonus* have been found throughout northern temperate areas of the world. (Thorn and Barron 1986) collected 713 samples from soil, rotting wood, mass, and other suitable nematode habitats yielded 49 isolates of *Nematoctonus*, representing five form species were *N. leisporus* (25 isolates) and *N.hamatus* (14 isolates) found most common. The best substrates for the isolation of *Nematoctonus*were were compost, with *Nematoctonus* isolated from 37.5% of the samples, followed by barnyard soil and dung(19.7%), sawmill wastes (15%), and rotting hardwoods (9.3%). The Baermann funnel technique yielded 29 isolates of *Nematoctonus*, the centrifuge technique 21 isolates, and soil sprinkling methods of *Nematoctonus* was recorded. Isolation of Nematoctonus species also depend upon the technique involved for its isolate i.e 5 isolates. The Baermann funnel and centrifuge technique were highly complementary, since only four isolates were duplicated by both technique. Four of the five isolates of *Nematoctonus* found by soil sprinkling method. Thus if time had been available for the routine use of soil sprinkling technique, considerably more isolates of *nematoctonus* might have been detected.

PREDACITY

The initial studies on predacity of these fungi were carried out mostly on saprophytic nematodes. Drechsler (1937, 1941) through his studies on predaceous fungi, gave detailed and precise information on predation of nematodes.

When nematodes come in contact with nematophagous fungi as a result of attraction or their free movement, both may exhibit different types of effect on each other during the course of interaction. Several experiments on interaction between nematophagous fungi and fungal feeder nematodes have been conducted by workers like (Cooke and Pramer 1968; Monoson 1968) to understand the ecology for better interpretation. Predation of Nematodes finally depends on the survival and growth of these fungi under a very diverse situation. Seeing the performance of these fungi, attention was diverted towards testing them against plant parasitic nematodes. Linford (1937) explored the possible use of these fungi as deterrent to plant parasitic nematodes causing diseases of plants. One of the major concerns was to find out if their capturing ability was general or specific to nematode species. Further the interest of workers clustered around whether the particular species of nematophagous fungi and nematode specie show best predation. A large number of workers have studied the predacity of nematophagous fungi against different species of plant parasitic nematodes. Only few of them studied the capturing ability of *Nematoctonus* against different species of plant parasitic nematodes and have reported their observations. (Dreschler, F.R. Jones, Giuma, & Cooke, (1971), Barron, (1977).

Giuma& Cooke (1971) studied production of toxin on nematodes by *N.robustus.* They suggested that spores of such fungi may quickly retard and stop their nematode hosts by production of toxin. These toxins stuck to the nematode cuticle, and before they are scraped off by the wriggling of the worm through the soil.

Barron (1977) describes *Nematoctonus*is one of the few genera of nematophagous fungi in which individual species may attack via mycelial traps or separate adhesive spores on nematodes so these may be termed as parasitoids.

The dilemma whether to screen isolates having more potential in the agar medium *in vitro* or in natural soil condition can be squeezed while testing the isolates which form directly trap structure or conidial trap either in the presence of soil or their extracts. Ashour (1999) observed that number of trap formation also depended upon the concentration of heavy metals. His results showed that maximum numbers of traps were produced by the fungus at 600 ppm of Zinc concentration and 200 ppm of Manganese while trap formation was greatly inhibited by all concentrations of Cadmium except 0.1 ppm. Anke et *al.*(1995) studied the Secondary metabolites with nematicidal and antimicrobial activity from nematophagous fungi and Ascomycetes. and identified *Nematoctonu srobustus* and *Nematoctonu sconcurrens* produced pleurotin, dihydropleurotinic acid, and leucopleurotin, metabolites previously isolated from cultures of *Hohenbuehelia* species, suggesting that the same biosynthetic pathways function in both the teleomorph and anamorph. Maciel*et al*. (2009) evaluated the *in vitro* effect of 12 isolates of fungus on the *Ancylostoma* spp. and found that *Nematoctonusrobustus* had the smallest percentages of reduction among the tested isolates and showed the lowest predaciousactivity. Silveira*et al.* (2009) isolated *Nematoctonuscampylosporus* from soil samples collected from different crops and agronomic environments in Brazil, using soil spreading methods. The nemato phagous activity of this fungus against free living nematodes (*Panagrellus* sp.) was documented using scanning electron microscopy.

MASS CULTURE

While studying the nematophagous fungi as potential biocontrol agents, it is important to produce mass culture of these fungi on various substrates. There are some commercial productions of these fungi as *A. robusta* var. "Antipolis" is marketed in France as "Royal 300" (Cayrol*et al.*, 1978). Another *A. irregularies*, is marketed in France as "Royal 350" for controlling root knot nematodes on tomato (Cayrol and Frankowski, 1979). Grewal and Sahi (1988) developed a technique for rapid multiplication of *A. conoides*involving soaking wheat grains in water for 20 min followed by 10-15 min. boiling and drying. Chalk and gypsum were mixed with culture and incubated at 24-26^{0}C. Guschin (1983) prepared stock culture of *A. oligospora* by using various food granules as filler. Boiling of the filler was found more reliable and economical than autoclaving. Tepyakova*et al.* (1993) developed a fungal preparation Nematofagin BL using two strains of *A. oligospora*, VKMF-2461 D and VKMF-3062 D, which was found effective against nematodes on vegetable crops. Matskievich (1993) also found Nematofagin BL to be effective against root knot nematodes in cucumbers, where 1.5 to 3.0 fold reduction of infestation over control was obtained. Matskievich (1990) developed technology for large scale production of *A. oligospora* on solid nutrient media such as composted straw manure, turf manure, saw dust manure and others.

BIOLOGICAL CONTROL

Nematode trapping behaviour on agar is well documented but observations in soil have been limited to those of Cook (1962 a, b, 1963), Kliejunas and Ko (1975) and Jansson (1982), who all used soil that were heavily amended with organic matter while Jaffee*et al.* (1992) used mineral soil.

Linford *et al.* (1938) carried out classical works on biological control of nematode by adding chopped, green pineapple tops to nematode infested soil in pots. They estimated the nematode population and activity of predatory fungi after application of pineapple tops and noted a marked increase in the number of free living nematodes in the soil. The increased population of nematode stimulated the population of predatory fungi that killed nematodes and brought them to below original level. Further Linford and Yap (1938, 1939) reported that out of five species of predacious fungi added to the soil, only few showed little control of nematodes. However, when these fungi were added along with organic matter, the biocontrol effect was much better. More or less similar results have been reported by various workers as reviewed by Duddington (1962) and Mankau (1980).

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